MOL2NET, International Conference Series on Multidisciplinary Sciences

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF ANTI-AMNESIC EFFECT OF *MORUS ALBA LINN* IN WISTAR RATS

Prathibha R 1[a], Manohari R 2 [a] ,Aswini S 3 [a], Saipriya Reddy 4 [a], Sandhya R 5 [a] ,Vasudha B 6 [a], Hemanth Kumar B 7 [a]*

[a] *Neuroscience R & D Center,Department of Pharmacology,School of Pharmacy,Anurag Group of Institutions,Hyderabad,Telangana,India.*

Abstract:

Back ground: Amnesia is defined as an abnormal mental state in which memory and learning are affected out of all proportion to other cognitive functions. Memory loss may result from two-sided damage to parts of the brain vital for memory storage, processing or recall. Benzodiazepines are known to produce anterograde amnesia by involvement of GABAergic system and by interference of long term potentiation (LTP).In this study, we aimed to evaluate the effect of *Morus Alba* fruit ethanolic extract (MAFEE) on diazepam induced amnesia in rats.

Materials and methods: *MAFEE* was administered for 14 successive days to rats. By using piracetam as the standard the ability of *MAFEE* on diazepam induced amnesia in Wistar rats was studied. The animals were randomly divided into 4 groups of Control, Diazepam treated (2 mg/kg), MAFEE (50 mg/kg) treated and Piracetam (200 mg/kg) treated respectively. Y-Maze and open field habituation were employed parameters. The Y-Maze and open field exploration tests were used to evaluate the anti –amnesic effect of MAFEE.

Result: Diazepam administration for 14 days to Wistar rats has induced anterograde amnesia.The parameters like percentage alternations(Y –Maze),line crossings, rearings and nose pokings (open field test) are used to assess the anti-amnesic effect of MAFEE in rats .Phytochemical investigation of MAFEE revealed the presence of both total phenolics (+++) and flavonoids (+++) which are responsible for antioxidant effects of MAFEE. After the administration of MAFEE the behavioral analysis in rats was performed using Y -Maze and Open Field tests .MAFEE administration in rats has shown a significant increase ($p<0.001$) in spontaneous

alternations on Y-Maze ,line crossings and rearing's on open field apparatus ($p<0.05$; $p<0.01$) when compared to diazepam treated group. However MAFEE administration has not shown any significant effect on nose poking's when compared to diazepam ($p>0.05$) treated group.

Conclusion: From our study results we validated the medicinal use of *Morus Alba* for its anti-amnesic effect but still further research at molecular level has to be initiated to evaluate its role in cognitive dysfunction.

Keywords: Y-Maze, line crossings, open field, Amnesia, Diazepam, piracetam.

Introduction:

In this 21st century the cognitive impairment and oxidative stress were most important functional aspects for studying the pathological outcomes of many neurodegenerative disorders such as Schizophrenia, Alzheimer's, Vascular dementia, cerebrovascular dysfunction, head injury/trauma and parkinsonism[1]. The cholinergic neuronal system plays a key role in learning and memory in humans and animals which is the rationale behind the use of nootropic drugs such as Piracetam and its analogues like oxiracetam and aniracetam[2]. However, several adverse effects associated with these drugs have limited their use as potent nootropics and search for newer agents and several clinical trials are still ongoing[2].

Plants and animal products have been an important basis for treatment of human diseases since ancient times in ayurveda.The mulberry belongs to the family Moraceae and genus Morus. The mulberry is a traditional edible fruit that is eaten fresh, or widely used in the production of wine, fruit juice; jam and canned food[3]. Earlier literature reports that mulberry fruit can protect against liver and kidney damage, strengthen the joints, improve eyesight and have anti-aging effects[4]. Mulberry fruit has also been used effectively in natural medicine for the treatment of sore throat, fever, hypertension and anemia [5]. Anthocyanin's and water extracts from mulberry fruit can scavenge free radicals, inhibit low-density lipoprotein (LDL) oxidation, and have beneficial effects on blood lipid and atherosclerosis [6]. As there were no earlier reports stating the pharmacological role of *Morus Alba* for its anti-amnesic activity. The present study was designed to evaluate its potential effect of MAFEE on diazepam-induced amnesia in Wistar rats.

Materials and Methods:

Animals:

Male wistar rats of 180-250g procured from National center for laboratory animal sciences, National institute of Nutrition, Hyderabad, India was used in this study.All animals were acclimatized for seven days before the experiment with free access to water *ad libitum* and standard laboratory pellet chow diet under constant temperature (22-24^{o}C humidity 45-50% on a 12 hour light and 12 hour dark cycle .the experiment was conducted between 9.00 and 18.00 hours under optimal conditions . The experiment protocol was approved by an institutional animal ethics committee of School of pharmacy, Anurag group of institutions and care of the animals was taken as per guidelines of the committee for the purpose of control and supervision of experiments on animals.**Reg no:1412/a/11/CPCSEA.**

Table 1. Drugs & Chemicals:

Chemicals	**Purchased from**
CMC(Carboxy methyl cellulose)	SD fine chemicals
Diazepam	Biochem pharma industries
Morus alba fruits	Medipally, HYD,Telangana
Piracetam	Dr.Reddy's laboratories

Authentification and collection of plant material:

Fresh ripe fruits of *Morus alba* were collected locally from Medipally Hyderabad, Telangana, India. The plant is identified and authenticated by Dr.B.Prathibha devi, Professor & Head, Department of Botany, Osmania University, Hyderabad, Telangana, India. A voucher specimen **(0334)** has been deposited in the herbarium department of the university and Pharmacognosy department of our institution.

Preparation of plant extract:

Dried fruits of *Morus Alba Linn* were mechanically powder and sieved. The coarsely powdered material (1000 g) was macerated with 10L of absolute ethanol for 1 week with occasional shaking. The extract was filtered and concentrated at reduced pressure on rotary evaporator resulting in dark brown colored mass.The yield of the final product during the extraction procedure was 7.32% (w/w). The residue was stored properly using air tight container in the refrigerator until further use.

Qualitative Phytochemical screening:

The qualitative phytochemical screening is performed for the plant extract to study or find out the primary and secondary metabolites present in it.

The phytochemical screening was carried out as two parts according to standard procedures [7] as follows:

1. Screening for primary metabolites
2. Screening for secondary metabolites.

Experimental design:

Group1 –Control:

In this group rats received CMC (1%) through oral route and the treatment was continued up to 14th day of the study.

Group 2-Negative Control:

In this group rats received Diazepam (2mg/kg) through intra peritoneal route and the treatment was continued up to 14th day of the study.

Group 3- Test:

In this group rats received MAFEE (50mg/kg) through oral route and the treatment was continued up to14th day of the study.

Group 4- Standard:

In this group the rats received Piracetam (200mg/kg) through intra peritoneal route and the treatment was continued up to 14th day of the study.

Table 2: Experimental design of the study

Groups	Name	Treatment
1	Control	CMC(1%w/v) was administered through oral gavage
2	Negative Control	Diazepam(2mg/kg/B.W) was administered through intraperitoneal injection
3	Test	MAFEE(50mg/kg/B.W) was administered through oral gavage
4	Standard	Piracetam (200mg/kg/B.W) was administered through intraperitoneal injection

Methods:

Y Maze Test:

Y-Maze spontaneous alternation is a behavioral test for measuring the willingness of rodents to explore new environments.Y-Maze is an apparatus that is made of gray plastic. Each arm is 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converged at an equal angle. Each rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. Rodents typically choose to investigate a new arm of the maze rather than returning to one that was previously visited. Many parts of the brain, including the hippocampus, septum, basal forebrain, and prefrontal cortex-are involved in this task. Alternation is defined as the successive entries into the three arms, on overlapping triplet sets. The ability to alternate requires that the rat know which arm they have already visited. The series of arm entries, including possible returns into the same arm, is recorded on the day 1 and day 2 using maze master software on video tracking system (VJ Instruments, Washim,Maharashtra, India). For each animal, the Y-maze testing was carried out for 5 min. The apparatus was cleaned with 5% alcohol and allowed to dry between sessions. The percentage alternation is calculated as {(actual alternations /maximum alternations) x 100} [8-12].

Open field test:

The exploratory behavior of the rat was evaluated by open field habituation task method. The arena of open field was divided into 16 squares- 4 inner squares and 12 squares in the periphery along the walls. After acclimatization to the lab and the open field apparatus, rats are placed individually in the center of the open field, the parameters which were observed for 5 minutes included the no. of squares crossed i.e. Line crossings ,rearing's and nose poking's [11,12].

Results:

Phytochemical investigation of MAFEE:

The MAFEE was subjected to qualitative chemical tests to determine the chemical constituents present and all the results were tabulated as below.

Table-3 :

Preliminary Phytochemical analysis of ethanolic extract of *Morus Alba* fruit:

S.NO.	Chemical Test		*Result*
1.	**Test for Carbohydrates** □ Molisch's Test (general test)		+++
	A.	**Test for Reducing Sugars**	
		□ Fehling's test	+++
		□ Benedicts test	+++
	B.	**Test for Monosaccharides**	
		□ Barfoed's test	-
2.	**Test for proteins**		
		□ Biuret test (General test)	-
		□ Millon's test	-
3.	**Test for Steroids**		
		□ Salkowski reaction	+++
		□Liebermann-Burchard	+++
4.	**Test for Flavonoids**		
		□ Shinoda test	+++
		□Alkaline reagent test	+++
5.	**Test for Alkaloids**		
		□ Dragendroff's test	-
		□ Mayer's test	-
		□ Hager's test	-
6.	**Test for phenolic compounds**		
		□ Ferric chloride test	+++
		□ Bromine water test	+++
		□ Dilute iodine test	+++

+++ve =Present; -ve=absent

Pharmacological investigation:

The *Morus Alba* fruit ethanolic extract was administered as 50mg/kg to evaluate its anti-amnesic effect.

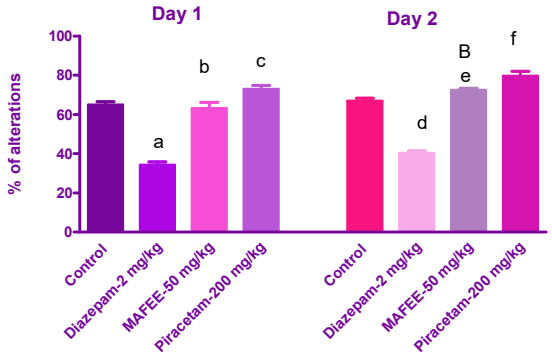
ANTI-AMNESIC ACTIVITY:

Effect of MAFEE treatment on Spontaneous alternations observed during Y-maze test :

Table-4: Effect of MAFEE administration on Day1 and Day2 spontaneous alternations observed during Y-maze test in Wistar rats.

Groups	Percentage alternations Observed on Day1	Percentage alternations Observed on Day 2
Control	64.83± 1.62	66.83± 1.57
Diazepam-2mg/kg	34.12± 1.76 [a]	36.33±1.58 [d]
MAFEE-50 mg/kg	67.17± 2.45 [b]	71.50±1.60 [e,B]
Piracetam-200 mg/kg	70± 2.35 [c]	73.33±2.01 [f]

Fig-1 : Effect of MAFEE administration on Day 1 and Day 2 Spontaneous alternations observed during Y-Maze test in Wistar rats



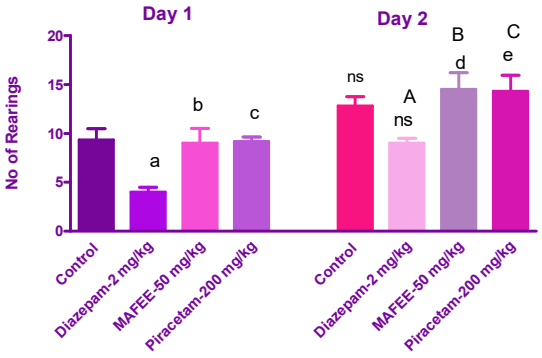
All data were expressed as Mean ± SEM using one way ANOVA followed by Bonferroni multiple comparison test. [B] P<0.01 when day 2 MAFEE-50 mg/kg is compared with day 1 MAFEE-50mg/kg treated group.[b,c,e,f,] P<0.001 when day1 & day2 MAFEE & Piracetam are compared with Diazepam treated group. [a,d] P<0.001)when day 1 & day 2 Diazepam is compared with Control

Open field activity:

Table-5: Effect of MAFEE administration on day 1 and day 2 rearing's during open field activity test in Wistar rats.

GROUP	Number of Rearing's observed on Day 1	Number of Rearing's observed on Day 2
Control	9.33±1.145	11.83± 1.352 [ns]
Diazepam-2mg/kg	4.00± 0.51 [a]	5.50±0.846 [A, ns]
MAFEE-50 mg/kg	8.83± 1.621 [b]	10.67±1.726 [d,B]
Piracetam-200 mg/kg	7.33± 0.91 [c]	9.00±0.856 [e, C]

Fig-2 : Effect of MAFEE administration on Day 1 and Day 2 Rearing's observed during Open Field activity test in Wistar rats

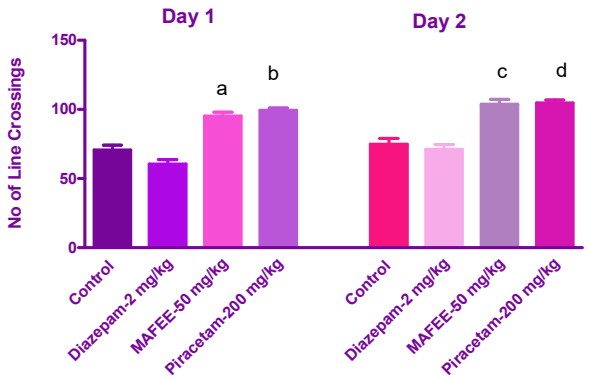


All data were expressed as Mean ± SEM using one way ANOVA followed by Bonferroni multiple comparison tests. ^{A} P <0.05 when day 2 Diazepam is compared with day 1 Diazepam treated group. ^{B} P<0.01 when day 2 MAFEE 50mg/kg was compared with day 1 MAFEE 50mg/kg treated group. ^{C} p<0.05 when day 2 piracetam is compared with day1 piracetam treated group. $^{b, c, d,e,}$ P<0.05 when day1 & day 2 MAFEE & Piracetam are compared with Diazepam treated groups. ^{a} P<0.05 when day 1 Diazepam was compared with Control group.^{ns} P<0.05 when day 2 Diazepam is compared with control group.

Table-6: Effect of MAFEE administration on Day1 and Day2 line crossings during open field activity

GROUP	Line crossings observed on Day1	Line crossings observed on Day 2
Control	70.67± 3.42	74.67± 4.31
Diazepam-2mg/kg	60.50± 3.13	63.33±3.43
MAFEE-50 mg/kg	95.00± 2.89 ^{a}	103.7±3.50 ^{c}
Piracetam-200 mg/kg	99.33± 1.62 ^{b}	104.5±2.27 ^{d}

Fig-3: Effect of MAFEE Administration on Day 1 and Day 2 Line crossings observed during Open Field Activity in wistar rats

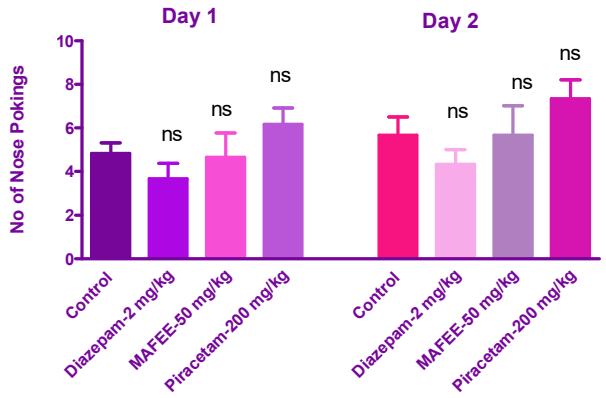


All data were expressed as Mean ± SEM using one way ANOVA followed by Bonferroni multiple comparision test.[a,b,c,d]P<0.001 when day1 & day 2 line crossings in MAFEE & Piracetam treated groups were compared with Diazepam treated group.

Table-7: Effect of MAFEE administration on Day 1 and Day 2 Nose poking's observed during open field activity in Wistar rats.

GROUP	Number of Nose poking's observed on Day 1	Number of Nose poking's observed on Day 2
Control	3.167±0.60	2.83± 0.60
Diazepam-2mg/kg	3.00± 0.57 ns	4.333±0.66 ns
MAFEE-50 mg/kg	4.667± 1.11 ns	5.67±1.35 ns
Piracetam-200 mg/kg	2.33± 0.49 ns	2±0.447 ns

Fig-4: Effect of MAFEE administration on Day 1 and Day 2 Nose Pokings observed during Open Field activity in Wistar rats



All data were expressed as mean ± SEM using One way ANOVA followed by Bonferroni multiple comparision test. nsP>0.05 (Non significant) when day 2 Diazepam, MAFEE, Piracetam were compared with day 1 Diazepam, MAFEE & Piroacetam treated groups.

Discussion:

The present study was designed to evaluate the anti-amnesic property of MAFEE on diazepam-induced amnesia model using exteroceptive behavioral models like Y-maze and open field tests on video tracking system. Wistar rats were chosen for this study as rodents are standardized experimental animals for behavioral study. Administration of diazepam for 14 days caused to cognitive impairment in rats, the present investigation reveals behavioral abnormalities in diazepam induced rats. Phytochemical tests revealed the presence of flavonoids, phenols, alkaloids & steroids. The main antioxidant activity of *Morus Alba* was mainly due to the presence of flavonoids & phenolic compounds.

Long-term potentiation (LTP) is a phenomenon responsible for the cellular mechanism of learning and memory processes. The Y-maze is a simple test for measuring spatial recognition memory that is based on discrimination of novelty versus familiarity in the three arms. The ability to alternate requires that the rats know which arm they have already visited. Supplementation with MAFEE improved percentage spontaneous alteration and indicates the escalation of LTP.

In this study spontaneous alternations in Y-Maze were calculated for all the groups. The CMC, Test & standard drugs were administered for 14 days & diazepam was given only on the 14^{th} day, and then behavioral assessment was carried out and a significant decrease in spontaneous alternations ($P<0.01$) with diazepam treated group is observed which revealed amnesia in rats. The significant increase in spontaneous alternations is noticed ($P<0.001$) when day 1 & day 2 MAFEE treated and Piracetam treated were compared with Diazepam treated groups respectively**.**

In behavioral study open field habituation memory was also carried out using open field test. It allowed the evaluation of habituation memory through measurement of the exploratory behavior (line crossings, rearings and nose

poking's). The treatment with MAFEE decreased the cognitive deficits in diazepam treated rats, especially; the data shown in open-field habituation memory with increase in the number of line crossings, rearing's but no significant effect was noticed with nose poking's. These results are consistent with the favorable effect on cognition in open-field habituation memory.MAFEE administration in rats has shown a significant increase ($p<0.001$) in line crossings and rearing's on open field apparatus ($p<0.05$; $p<0.01$) when compared to diazepam treated group. However MAFEE administration has not shown any significant effect on nose poking's when compared to diazepam ($p>0.05$) treated group.

In both the models MAFEE showed significant amelioration of behavioral activity, when compared to diazepam treated animals. Earlier literature also suggest that different extracts of *Morus Alba* plant has been proven for their rich antioxidant, neuroprotective, anxiolytic, antidiabetic ,analgesic, hepatoprotective ,hypotensive,hyperlipedemic and vascular protective activities [13-21] but no earlier reports on fruit ethanolic extract antiamnesic activity which was validated by our study. To summarize, the present data indicates that the administration of MAFEE extract showed significant anti-amnesic activity, mediated centrally and peripherally.This may be attributed to flavonoids and phenolic compounds in the extract analyzed during the phytochemical screening of MAFEE. However further study has to be initiated to understand the precise mechanism of Anti-amnesic effect exhibited by MAFEE.

Conclusion:

In conclusion, This study reveals the potential of *Morus Alba* as anti-amnesic however there is a need for further evaluation of different fractions of ethanolic extracts *Morus Alba* fruit using various biochemical and histopathological studies and evaluate *Morus alba* as a potential remedy for the treatment of amnesia.

References *(mandatory)*

1. Ingole, S.R., Satyendra, K., Sharma, R., Sharma, S.S., (2008). Cognition Enhancer: Current Strategies and Future Perspectives, *Current Research & Information on Pharmaceutical Sciences*, 9, 42-48.
2. Joshi, H., Kaur, N., Chauhan, J., (2007). Evaluation of Nootropic Effect of A. specicosa in Mice, *Journal of Health Science*, 53(4), 382-388.
3. Ning, DW., Lu, B., Zhang, YL.,(2005). The processing technology of mulberry series product.*China Fruit Vegetables Process*, 5: 38-40.
4. S.Z. Li., (1982). Compendium of Materia Medica, *People's Medical Press*, Beijing, 2066–2067.
5. S.X. Gong., J.P. Zhu., (2008). Mulberry relieving nutritional anemia, *J. Zhejiang Univ.Trad. Chinese Medicine*, 32(3),350–352.
6. Q. Du., J. Zheng., Y. Xu .,(2008) .Composition of anthocyanins in mulberry and their antioxidant activity", *Journal of Food Composition and Analysis*, 21,390–395.
7. Rajput, M.A., & Khan, R.A., (2017). Phytochemical screening, acute toxicity, anxiolytic and antidepressant activities of the *Nelumbo nucifera* fruit. *Metabolic Brain Disease*, 32: 743.
8. Hemanth Kumar, B., Arun Reddy, R., Mahesh Kumar, J., Dinesh Kumar, B., Diwan P.V., (2017). Effects of fisetin on hyperhomocysteinemia-induced experimental endothelial dysfunction and vascular dementia. *Can. J. Physiol. Pharmacol*, 9(1),32-42. doi: 10.1139/cjpp-2016-0147.
9. Hemanth Kumar, B., Dinesh Kumar, B., Diwan, P.V.,(2017) . Hesperidin, a citrus flavonoid, protects against l-methionine-induced hyperhomocysteinemia by abrogation of oxidative stress, endothelial dysfunction and neurotoxicity in Wistar rats. *Pharm. Biol*, 55(1),146-155. doi:10.1080/13880209.2016.1231695.
10. Hemanth Kumar, B., Mahesh Kumar, J., Dinesh Kumar, B., Diwan, PV., (2018). Influence of fisetin combined with hesperidin on chronic mild hyperhomocysteinemia induced cognitive dysfunction and oxidative stress in wistar rats, *PharmaNutrition*, 6(3),125-136,doi.org/10.1016/j.phanu.2018.06.003.
11. Singh, J.C., Kakalij, R.M., Kshirsagar, R.P., Kumar, B.H., Komakula, S.S., Diwan, P.V., (2015).Cognitive effects of vanillic acid against streptozotocin-induced neurodegeneration in mice. *Pharmaceutical Biology*, 53 (5),630-6. doi: 10.3109/13880209. 2014.935866.
12. Vinitha, E., Hanish, J.C.S., Rahul, M.K., Rahul, P.K., Boyina, H.K., Diwan, P.V., (2014). Neuroprotective effect of Prunus avium on streptozotocin induced neurotoxicity in mice, *Biomedicine & Preventive Nutrition*,4(4),519-525,doi.org/10.1016/j.bionut.2014.08.004.

13. Kim, D. S., Kang, Y. M., Jin, W. Y., Sung, Y. Y., Choi, G., & Kim, H. K. (2014). Antioxidant activities and polyphenol content of Morus alba leaf extracts collected from varying regions. *Biomedical reports*, 2(5), 675-680.
14. Zhang, H., Ma, Z. F., Luo, X., & Li, X. (2018). Effects of Mulberry Fruit (Morus alba L.) Consumption on Health Outcomes: A Mini-Review. *Antioxidants* (Basel, Switzerland), 7(5), 69. doi:10.3390/antiox7050069
15. Wang, Y., Xiang, L., Wang, C., Tang, C., & He, X. (2013). Antidiabetic and antioxidant effects and phytochemicals of mulberry fruit (Morus alba L.) polyphenol enhanced extract. *PloS one*, 8(7), e71144. doi:10.1371/journal.pone.0071144
16. Khan, M. A., Rahman, A. A., Islam, S., Khandokhar, P., Parvin, S., Islam, M. B., Hossain, M., Rashid, M., Sadik, G., Nasrin, S., Mollah, M. N., Alam, A. H. (2013). A comparative study on the antioxidant activity of methanolic extracts from different parts of Morus alba L. (Moraceae). *BMC research notes*, 6, 24. doi:10.1186/1756-0500-6-24
17. Seo, K.H., Lee, D.Y., Jeong, R.H., Lee, D.S., Kim, Y.E., Hong, E.K., et al. (2015). Neuroprotective effect of prenylated arylbenzofuran and flavonoids from morus alba fruits on glutamate-induced oxidative injury in HT22 hippocampal cells. *J Med Food*, 18(4), 403–8. doi: 10.1089/jmf.2014.3196.
18. Jiao, Y., Wang, X., Jiang, X., Kong, F., Wang, S., Yan, C.,(2017). Antidiabetic effects of Morus alba fruit polysaccharides on high-fat diet- and streptozotocin-induced type 2 diabetes in rats. *J Ethnopharmacol*, 199,119–27. doi: 10.1016/j.jep.2017.
19. Yadav, A. V., Kawale, L. A., & Nade, V. S. (2008). Effect of Morus alba L. (mulberry) leaves on anxiety in mice. *Indian journal of pharmacology*, 40(1), 32-6.
20. Jung, J.W., Ko, W.M., Park, J.H., Seo, K.H., Oh, E.J., Lee, DY., et al. (2015). Isoprenylated flavonoids from the root bark of Morus alba and their hepatoprotective and neuroprotective activities. *Arch Pharm Res*, 38(11), 2066–75. doi: 10.1007/s12272-015-0613-8.
21. Lee, Y.J., Choi, D.H., Kim, E.J., Kim, H.Y., Kwon, T.O., Kang, D.G., et al. (2011). Hypotensive, hypolipidemic, and vascular protective effects of Morus alba L. in rats fed an atherogenic diet. *Am J Chin Med*, 39(1), 39–52. doi: 10.1142/s0192415x11008634.